



2850-106.ST25
SEQUENCE LISTING

<110> Yokoyama, Shigeyuki
Shirouzu, Mikako
Sakamoto, Ayako
Sakamoto, Kensaku

<120> METHOD OF EXPRESSING PROTEINS COMPRISING NON-NATURALLY-OCCURRING
AMINO ACIDS (As Amended)

<130> P/2850-106

<140> 10/532,948

<141> 2005-11-10

<150> PCT/JP03/14028

<151> 2003-10-31

<150> 2002-318846

<151> 2002-10-31

<160> 34

<170> PatentIn version 3.3

<210> 1

<211> 167

<212> DNA

<213> Artificial

<220>

<223> an artificial base sequence consisting of a leader sequence of
human tRNA gene, and the tRNA^{tyr} gene of B. stearothermophilus
with a CUA anticodon, but without the terminal CCA sequence and a
transcription terminator

<400> 1

agcgctccgg tttttctgtg ctgaacctca ggggacgccg acacacgtac acgtcggagg 60

ggtagcgaag tggctaaacg cggcggactc taaatccgct ccctttgggt tcggcggttc 120

gaatccgtcc ccctccagac aagtgcggtt tttttctcca gctccc 167

<210> 2

<211> 44

<212> DNA

<213> Artificial

<220>

<223> a PCR primer used for amplifying a part of genomic DNA of E. coli

<400> 2

ggaattccat atggcaagca gtaacttgat taaacaattg caag 44

<210> 3

<211> 50

<212> DNA

<213> Artificial

<220>

<223> a PCR primer used for amplifying a part of genomic DNA of E.

coli.

<400> 3
gccgaagctt gtcgactttc cagcaaatca gacagtaatt ctttttaccg 50

<210> 4
<211> 40
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 4
aggatcgaag ccgcaagcga gcgcgatcgg gccttgcgcc 40

<210> 5
<211> 40
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<220>
<221> misc_feature
<222> (16)..(16)
<223> m represents c or a

<220>
<221> misc_feature
<222> (17)..(18)
<223> n is a, c, g, or t

<400> 5
aggatcgaag ccgcamnga gcgcgatcgg gccttgcgcc 40

<210> 6
<211> 33
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 6
acggtgtggt gctgtctatt ggtggttctg acc 33

<210> 7
<211> 33
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

invention

<400> 7
acggtgtggt gctggcaatt ggtggttctg acc 33

<210> 8
<211> 33
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 8
acggtgtggt gctgaacatt ggtggttctg acc 33

<210> 9
<211> 33
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 9
acggtgtggt gctgtgcatt ggtggttctg acc 33

<210> 10
<211> 32
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 10
ttcttcggat ccaaccagac tgcgccgct tc 32

<210> 11
<211> 30
<212> DNA
<213> Artificial

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 11
gatcatctgg ttaacggaga agtgtttgcc 30

<210> 12
<211> 26
<212> DNA
<213> Artificial

2850-106.ST25

<220>
<223> a PCR primer used in the overlapping extension in the present invention

<400> 12
gaccttcctg tgcgatattg gcaaac

26

<210> 13
<211> 12
<212> DNA
<213> Artificial

<220>
<223> the box A consensus sequence

<220>
<221> misc_feature
<222> (2)..(2)
<223> r represents g or a

<220>
<221> misc_feature
<222> (5)..(6)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (10)..(10)
<223> n is a, c, g, or t

<400> 13
trgcnnagyn gg

12

<210> 14
<211> 11
<212> DNA
<213> Artificial

<220>
<223> the box B consensus sequence

<220>
<221> misc_feature
<222> (8)..(8)
<223> n is a, c, g, or t

<400> 14
ggttcgantic c

11

<210> 15
<211> 20
<212> DNA
<213> Artificial

<220>
<223> a sequence of a primer binding site pbs1

<400> 15

	2850-106.ST25	
agcgagtgtt aaccctgcct		20
<210> 16		
<211> 20		
<212> DNA		
<213> Artificial		
<220>		
<223> a sequence of a primer binding site pbs2		
<400> 16		
cgactacgat attcgcgag		20
<210> 17		
<211> 12		
<212> DNA		
<213> Artificial		
<220>		
<223> a sequence of a BstXI-1 site		
<400> 17		
ccagcagact gg		12
<210> 18		
<211> 12		
<212> DNA		
<213> Artificial		
<220>		
<223> a sequence of a BstXI-2 site		
<400> 18		
ccagcttcct gg		12
<210> 19		
<211> 63		
<212> DNA		
<213> Artificial		
<220>		
<223> a nucleotide sequence coding a short peptide used for substitution of green fluorescent protein (cyanfluorescent mutation)		
<400> 19		
atgggaacta gtccatagtg gtggaattct gcagatatcc agcacagtgg cggccgccgc		60
gtc		63
<210> 20		
<211> 11		
<212> DNA		
<213> Artificial		
<220>		
<223> another box B consensus sequence		

<220>
 <221> misc_feature
 <222> (8)..(8)
 <223> n is a, c, g, or t

 <400> 20
 agttcgantc t 11

 <210> 21
 <211> 31
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer used for amplifying the sequence of SEQ ID No. 1

 <400> 21
 cacagaattc tcgggagctg gagaaaaaaa c 31

 <210> 22
 <211> 30
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of another primer used for amplifying the sequence of SEQ ID No. 1

 <400> 22
 cacaaagctt agcgctccgg tttttctgtg 30

 <210> 23
 <211> 40
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment having a primer binding site pbs1 upstream of the sequence of SEQ ID No. 1 and BstXI-1 site downstream thereof

 <400> 23
 agcgagtgtt aaccctgcct agcgctccgg tttttctgtg 40

 <210> 24
 <211> 38
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment having a primer binding site pbs1 upstream of the sequence of SEQ ID No. 1 and BstXI-1 site downstream thereof

 <400> 24
 acacaccag cagactggcg ggagctggag aaaaaaac 38

2850-106.ST25

<210> 25
 <211> 38
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment having
 a BstXI-1 site upstream of the sequence of SEQ ID No. 1 and
 another BstXI-1 site downstream from the first BstXI-1 site

 <400> 25
 acacaccag cagactggag cgctccggtt tttctgtg 38

 <210> 26
 <211> 38
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment having
 a BstXI-1 site upstream of the sequence of SEQ ID No. 1 and
 another BstXI-1 site downstream from the first BstXI-1 site

 <400> 26
 acacaccag cttcctggcg ggagctggag aaaaaaac 38

 <210> 27
 <211> 38
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment having
 a BstXI-2 site upstream of the sequence of SEQ ID No. 1 and a
 primer binding site pbs-2

 <400> 27
 acacaccag cttcctggag cgctccggtt tttctgtg 38

 <210> 28
 <211> 40
 <212> DNA
 <213> Artificial

 <220>
 <223> a sequence of a primer set used for amplifying a fragment hving a
 BstXI-2 site upstream of the sequence of SEQ ID No. 1 and a
 primer binding site pbs-2

 <400> 28
 ctgcgcgaat atcgtagtcg cgggagctgg agaaaaaac 40

 <210> 29
 <211> 424
 <212> PRT
 <213> Escherichia coli

 <400> 29

2850-106.ST25

Met Ala Ser Ser Asn Leu Ile Lys Gln Leu Gln Glu Arg Gly Leu Val
 1 5 10 15
 Ala Gln Val Thr Asp Glu Glu Ala Leu Ala Glu Arg Leu Ala Gln Gly
 20 25 30
 Pro Ile Ala Leu Tyr Cys Gly Phe Asp Pro Thr Ala Asp Ser Leu His
 35 40 45
 Leu Gly His Leu Val Pro Leu Leu Cys Leu Lys Arg Phe Gln Gln Ala
 50 55 60
 Gly His Lys Pro Val Ala Leu Val Gly Gly Ala Thr Gly Leu Ile Gly
 65 70 75 80
 Asp Pro Ser Phe Lys Ala Ala Glu Arg Lys Leu Asn Thr Glu Glu Thr
 85 90 95
 Val Gln Glu Trp Val Asp Lys Ile Arg Lys Gln Val Ala Pro Phe Leu
 100 105 110
 Asp Phe Asp Cys Gly Glu Asn Ser Ala Ile Ala Ala Asn Asn Tyr Asp
 115 120 125
 Trp Phe Gly Asn Met Asn Val Leu Thr Phe Leu Arg Asp Ile Gly Lys
 130 135 140
 His Phe Ser Val Asn Gln Met Ile Asn Lys Glu Ala Val Lys Gln Arg
 145 150 155 160
 Leu Asn Arg Glu Asp Gln Gly Ile Ser Phe Thr Glu Phe Ser Tyr Asn
 165 170 175
 Leu Leu Gln Gly Tyr Asp Phe Ala Cys Leu Asn Lys Gln Tyr Gly Val
 180 185 190
 Val Leu Gln Ile Gly Gly Ser Asp Gln Trp Gly Asn Ile Thr Ser Gly
 195 200 205
 Ile Asp Leu Thr Arg Arg Leu His Gln Asn Gln Val Phe Gly Leu Thr
 210 215 220
 Val Pro Leu Ile Thr Lys Ala Asp Gly Thr Lys Phe Gly Lys Thr Glu
 225 230 235 240
 Gly Gly Ala Val Trp Leu Asp Pro Lys Lys Thr Ser Pro Tyr Lys Phe
 245 250 255

2850-106.ST25

Tyr Gln Phe Trp Ile Asn Thr Ala Asp Ala Asp Val Tyr Arg Phe Leu
260 265 270

Lys Phe Phe Thr Phe Met Ser Ile Glu Glu Ile Asn Ala Leu Glu Glu
275 280 285

Glu Asp Lys Asn Ser Gly Lys Ala Pro Arg Ala Gln Tyr Val Leu Ala
290 295 300

Glu Gln Val Thr Arg Leu Val His Gly Glu Glu Gly Leu Gln Ala Ala
305 310 315 320

Lys Arg Ile Thr Glu Cys Leu Phe Ser Gly Ser Leu Ser Ala Leu Ser
325 330 335

Glu Ala Asp Phe Glu Gln Leu Ala Gln Asp Gly Val Pro Met Val Glu
340 345 350

Met Glu Lys Gly Ala Asp Leu Met Gln Ala Leu Val Asp Ser Glu Leu
355 360 365

Gln Pro Ser Arg Gly Gln Ala Arg Lys Thr Ile Ala Ser Asn Ala Ile
370 375 380

Thr Ile Asn Gly Glu Lys Gln Ser Asp Pro Glu Tyr Phe Phe Lys Glu
385 390 395 400

Glu Asp Arg Leu Phe Gly Arg Phe Thr Leu Leu Arg Arg Gly Lys Lys
405 410 415

Asn Tyr Cys Leu Ile Cys Trp Lys
420

<210> 30
<211> 135
<212> DNA
<213> Artificial

<220>
<223> a sequence of one of the induced expression systems prepared in
Example 2 (TetBst0)

<400> 30
tctccctatc agtgatagag atcggagggg tagcgaagtg gctaaacgcg gcggactcta 60
aatccgctcc ctttgggttc ggcggttcga atccgtcccc ctccagacaa gtgcggtttt 120
tttctccagc tcccg 135

2850-106.ST25

<210> 31
 <211> 145
 <212> DNA
 <213> Artificial

<220>
 <223> a sequence of one of the induced expression systems prepared in Example 1 (TetBst1)

<400> 31
 tctccctatc agtgatagag atccgtacac gtcggagggg tagcgaagtg gctaaacgcg 60
 gcggactcta aatccgctcc ctttgggttc ggcgggttcga atccgtcccc ctccagacaa 120
 gtgcggtttt tttctccagc tcccg 145

<210> 32
 <211> 155
 <212> DNA
 <213> Artificial

<220>
 <223> a sequence of one of the induced expression systems prepared in Example 2 (TetBst2)

<400> 32
 tctccctatc agtgatagag atccgccgac acacgtacac gtcggagggg tagcgaagtg 60
 gctaaacgcg gcggactcta aatccgctcc ctttgggttc ggcgggttcga atccgtcccc 120
 ctccagacaa gtgcggtttt tttctccagc tcccg 155

<210> 33
 <211> 85
 <212> DNA
 <213> Escherichia coli

<400> 33
 ggtgggggttc ccgagcggcc aaaggagca gactctaaat ctgccgtcac agacttcgaa 60
 ggttcgaatc cttccccccac cacca 85

<210> 34
 <211> 85
 <212> DNA
 <213> Bacillus stearothermophilus

<400> 34
 ggaggggtag cgaagtggct aaacgcggcg gactctaaat ccgctccctt tgggttcggc 60
 ggttcgaatc cgtccccctc cacca 85